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Forestry Researchers:

help lift the "veil of secrecy" on the causes of tree disease

photo story no. 27

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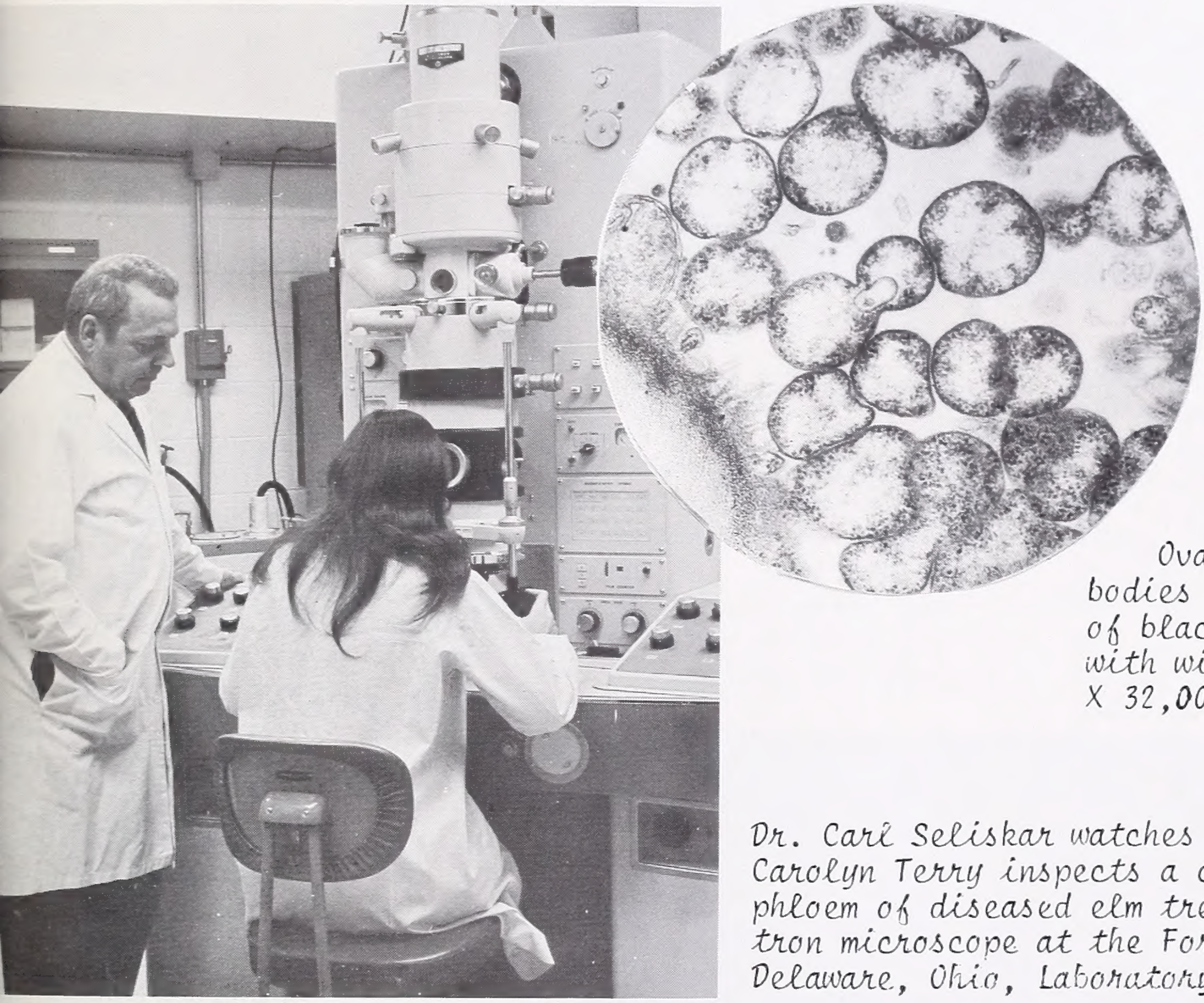

Oval mycoplasmalike bodies inside the phloem of black locust affected with witches'-broom. X 32,000.

Dr. Carl Seliskar watches as Lab Technician Carolyn Terry inspects a cross section of phloem of diseased elm tree under the electron microscope at the Forest Service's Delaware, Ohio, Laboratory.

The cause of many diseases--regardless of whether they attack humans, animals, or plants--are cloaked in mystery. But it is a known fact that viruses, bacteria, and mycoplasmas (a specialized form of bacteria) cause diseases in humans and animals, and that viruses and bacteria cause diseases in plants.

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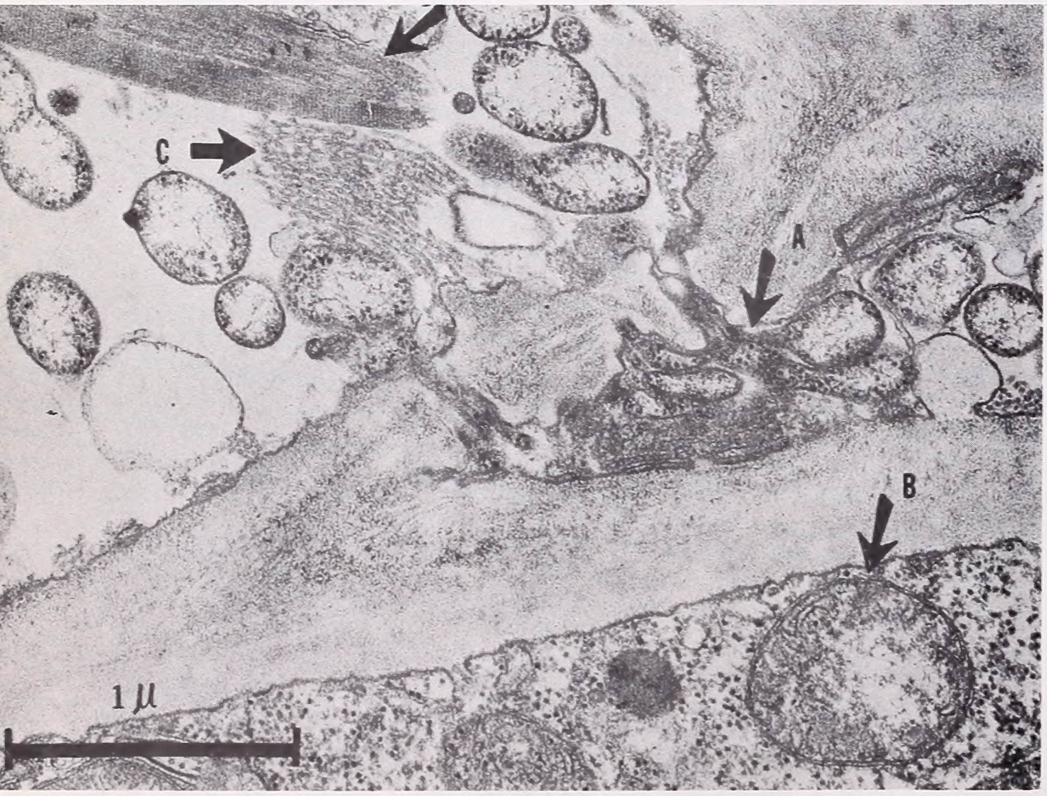
Advanced stages of elm phloem necrosis in American elm, left. Leaves are small, chlorotic, and curved inward. Trees die in 1-3 years.

Below, mycoplasmalike bodies (A) passing through a sieve plate are seen as narrow tubular bodies. Other elements in the cell are: mitochondrion (B); P-protein (C); and crystalline P-protein (D).

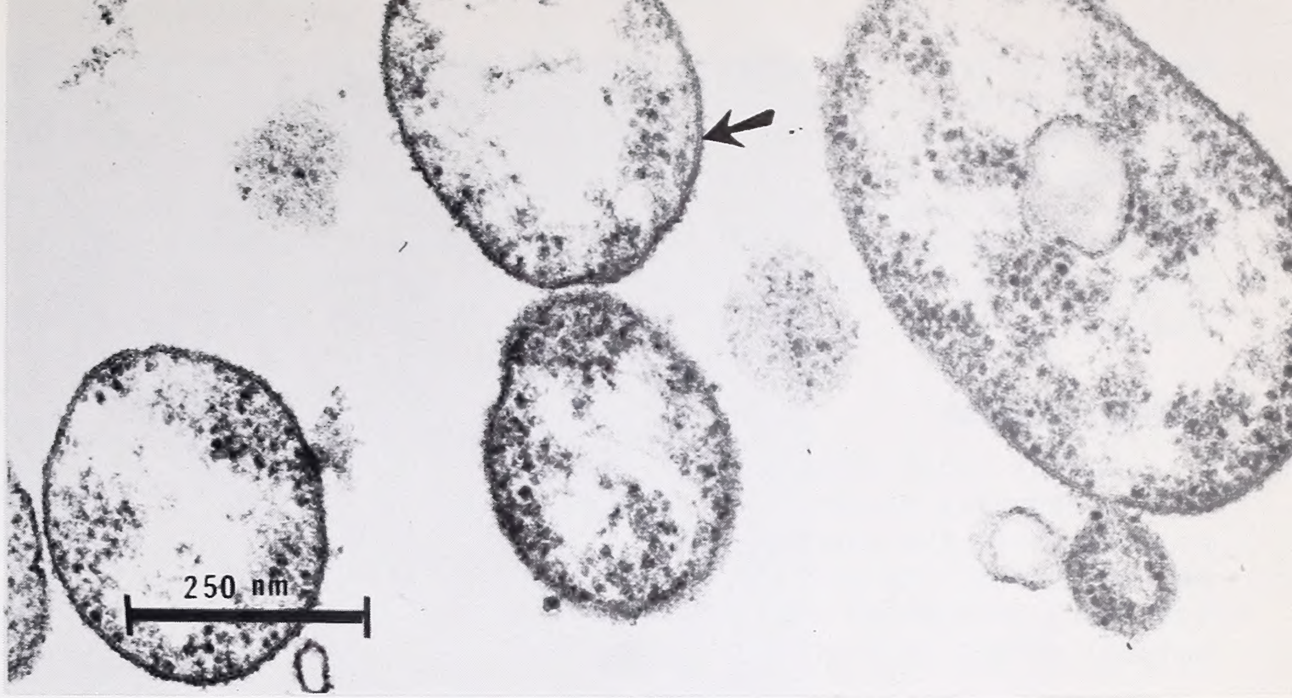


link discovered

In 1967, Japanese scientists reported also discovering a link between some plant diseases and mycoplasmas. This finding spurred a great deal of research, and now mycoplasmas are known to be associated with over 50 yellow disorders in plants (Yellow disorders are characterized by an overall yellowing and dwarfing of foliage, or by the development of witches'-brooms in the crown of trees.)

Forest Service scientists, involved in investigating several yellows-type diseases, recently took on mycoplasma studies as part of their work.

In studies headed by Dr. Carl E. Seliskar, a plant pathologist at the Forest Insect and Disease Laboratory in Delaware, Ohio, the Forest Service scientists were able to



A three-layered unit membrane (arrow) of the mycoplasmalike body in diseased locust is evident in this electron micrograph.

confirm the relationship of mycoplasmalike bodies to at least four yellows-type tree diseases--black locust, witches'-broom, pecan bunch, elm phloem necrosis, and walnut bunch.

Mycoplasmas are most often referred to as mycoplasmalike bodies in plant tissue. Found in the phloem tissue of some diseased trees, they very closely resemble the mycoplasmas found in diseased animal tissue.

Because they contain no rigid cell wall they may assume various shapes. Most commonly they are oval or nearly spherical in shape, but filamentous forms are not uncommon. This form seems to occur when they are ready to leave one cell to enter into another via a sieve plate.

They appear to divide by fission. Although they occur in a great variety of sizes--from 80-1000 nm--most appear to be between 200-350 nm. They are invisible to the naked eye and can only be seen under an electron microscope.

In extensive testing, Dr. Seliskar found mycoplasmalike bodies present in the phloem of black locust trees affected with witches'-broom; however, these bodies were completely absent from the phloem tissue of healthy black locust trees.

The same phenomenon was observed among pecan and walnut trees: those that were infected with pecan and walnut bunch diseases had mycoplasmalike bodies in their phloem tissue; those that were healthy did not.

black locust broom

Black locust witches broom was described in 1898 as a viruslike disorder, because it could be transmitted by grafting. At that time it was found only in Maryland, but it has since spread throughout the United States and into several European countries.

The disease results in systematic brooming of root and stump sprouts, roots and sometimes crowns of affected trees. Brooms are produced by the abnormal growth of normally dormant leaf buds. Brooms form late in summer and continue to grow late into fall. They frequently die during the winter.

After collecting diseased black locust branches, roots, and root sprouts with various degrees of brooming during two successive years and comparing these with healthy nursery stock, the

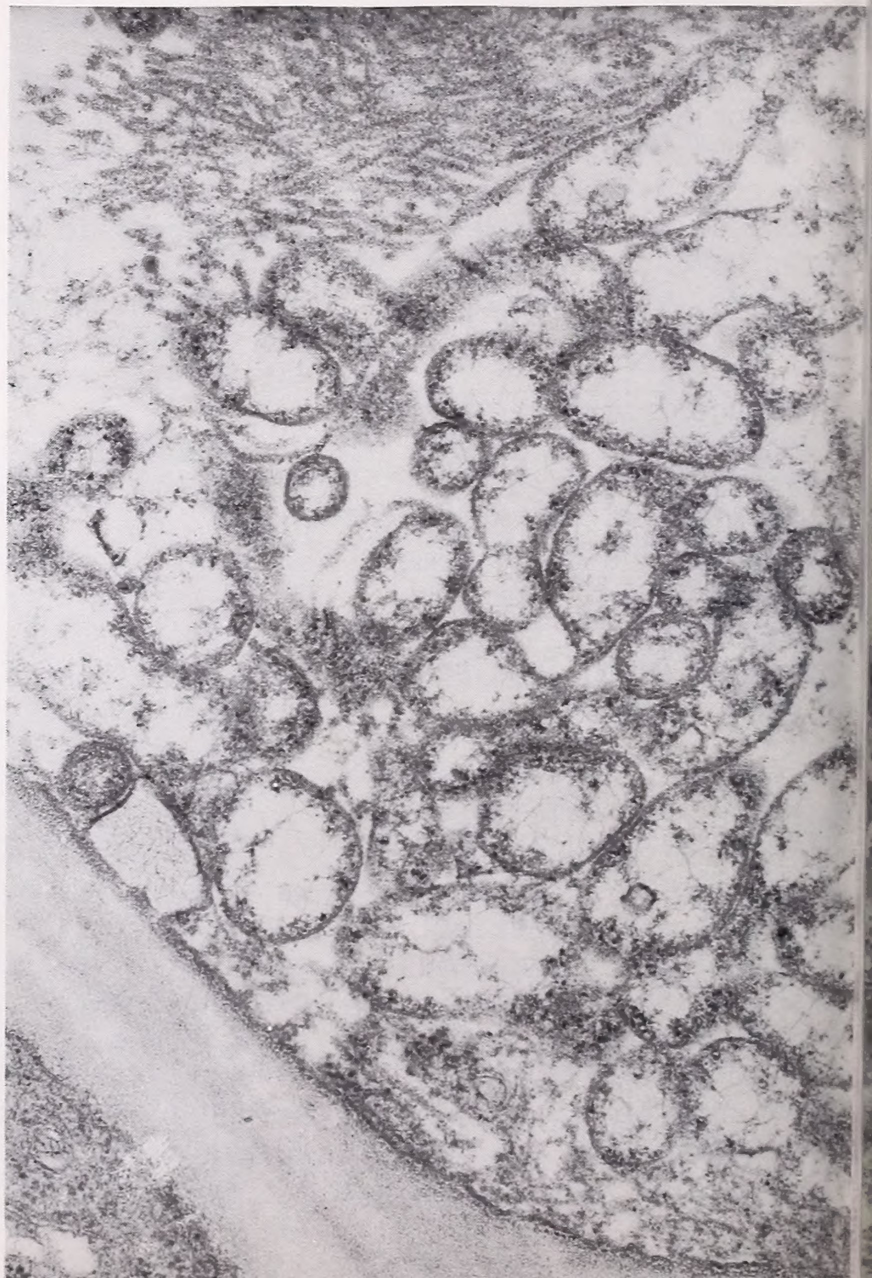
Forest Service scientists prepared thin sections of tissue for examination by electron microscope. This led to their confirming the relationship of black locust witches'-broom and mycoplasmas.

walnut disease

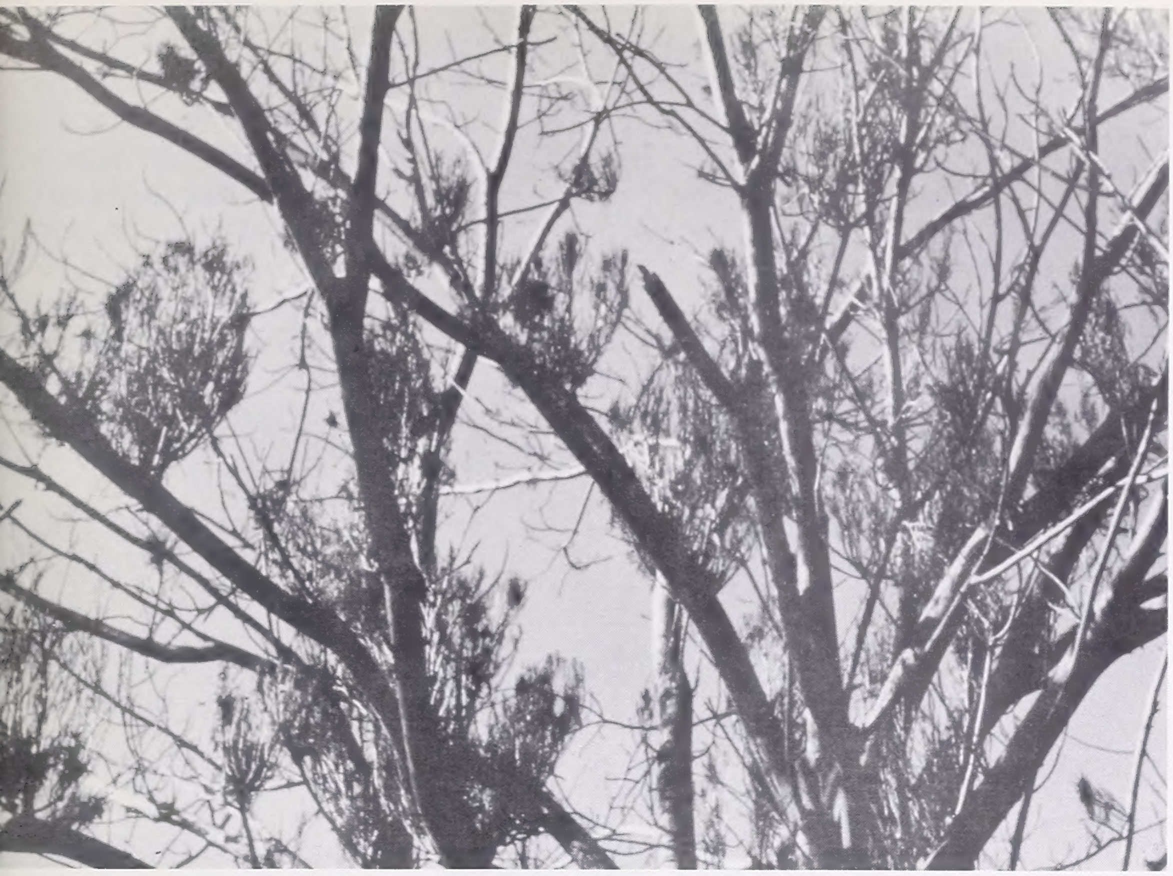
Walnut bunch disease is a widespread disease of walnuts in the eastern United States, and probably occurs throughout the natural ranges of black walnut and butternut. The disease also infects Japanese and Persian walnuts and has caused serious losses in Eastern nut orchards for some 60 years. The disease has practically eliminated Japanese walnut in the East but it is less serious on other susceptible species, particularly on black walnut where symptoms are sometimes masked.

Symptoms first appear as small brooms which arise from the premature development of normally dormant axillary buds. Brooms begin growth in early spring before normal foliage develops and are easily spotted at this time. Also, they continue development late in the fall after normal leaves have been shed.

Like most yellows-type diseases, walnut bunch is systemic but its rate of movement within the tree is often very slow. Like most yellows-



Typical mycoplasmalike bodies in sieve element of walnut infected with bunch disease. Some bodies appear to be dividing by fission. Strands of fibrillar material, probably DNA, appear in most bodies. X 46,000.



Numerous brooms are shown on this Japanese Walnut infected with bunch disease.

type diseases, walnut bunch was presumed to be virus-caused when it was transmitted by grafting. However, since the recent discovery of mycoplasmas in association with certain yellows-type disorders, a comprehensive search for mycoplasma in walnut bunch disease has been made.

Eventually electron microscope search proved fruitful. Mycoplasma were found in the phloem tissues in the petioles, roots, and stems of diseased trees. They were most abundant in petioles of highly symptomatic twigs in brooms and rarely found in non-symptomatic portions of diseased trees. Spherical or oval shapes were most common but tubular forms were also present, especially near the sieve plates. Thus far, attempts to grow the mycoplasma organism in culture have failed.

pecan bunch

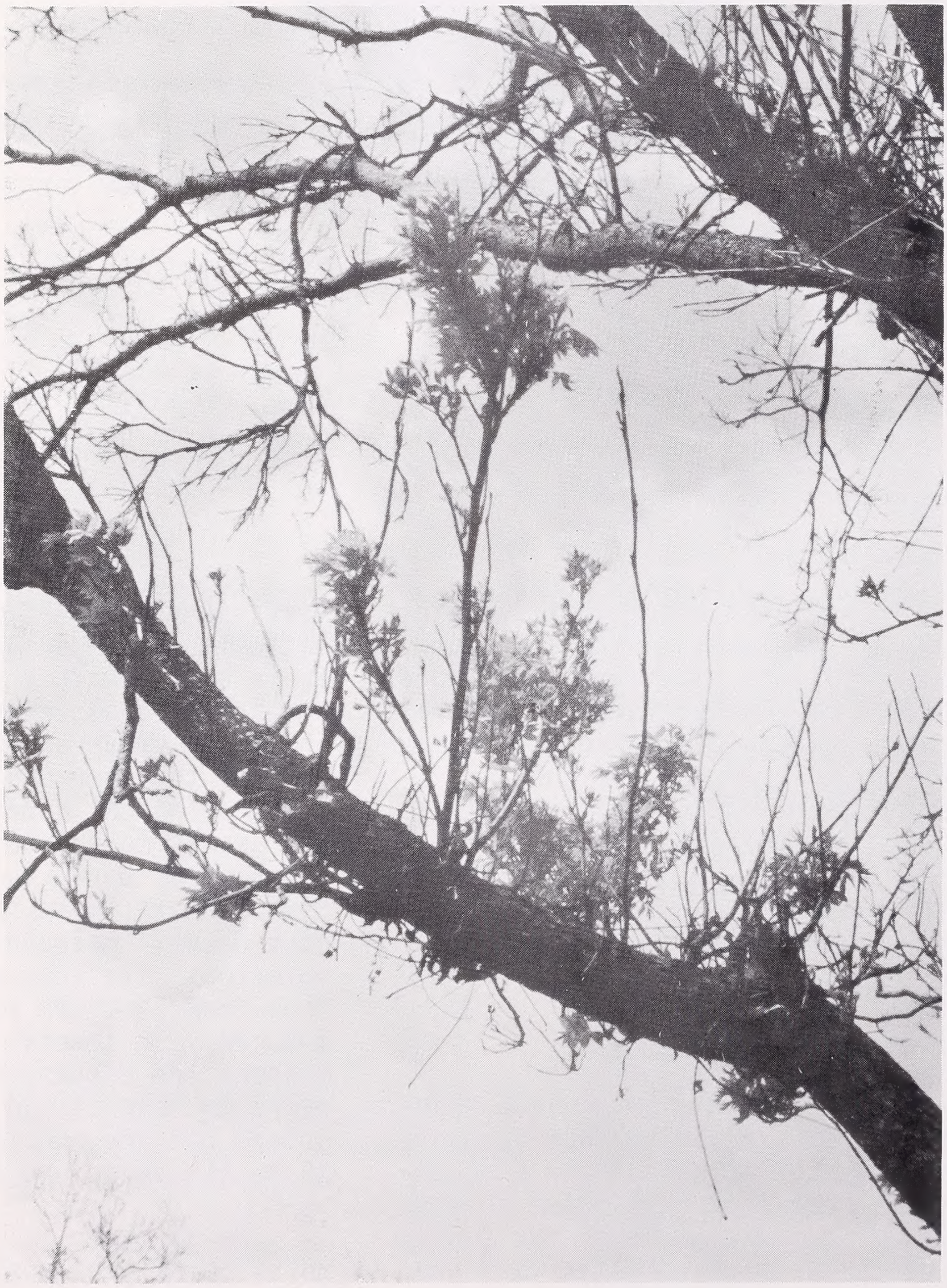
Pecan bunch is a widespread and serious disorder that occurs in the 8 southern states that are in the commercial range for southern pecan. It is most severe among pecans in the bottomlands of Louisiana. It is believed that the disease spreads initially from wild pecan and water hickory to commercial pecan orchards, since the disease is most severe in orchards bordering on bayous where these species occur. Being highly infectous, it, too, was long thought to be virus-induced. In several instances the disease appears to have spread from upland hickory species to nearby pecan orchards.

Pecan bunch is not so systematic as most virus diseases and some other yellows-type diseases. Instead, it spreads quite slowly within the tree.

Like walnut bunch, it may occur on one branch or part of a tree for years before its symptoms appear anywhere else on that tree, if, in fact, they occur elsewhere at all. There is evidence that this disease may be eradicated from mildly diseased pecans by pruning limbs well below their brooms.

Pecan bunch produces many brooms with few to many slender shoots on trunk and branches. These shoots leaf out before normal foliage in spring, continue to grow late in the fall, and then are often killed during the winter.

Forest Service scientists collected diseased and healthy leaf and twig materials from several orchards in Louisiana. They



In bunch disease of pecan the brooms begin growth in early spring before normal foliage develops and are easily spotted at this time.

next prepared tissues for examination with an electron microscope. They found the mycoplasmas of pecan bunch similar in appearance to those found in black locust witches'-broom and walnut bunch disease.

other diseases

In further testing, Dr. Seliskar and his team of Forest Service scientists parasitized a number of flowering periwinkle plants with dodder, a leafless vinelike plant.

After the dodder became established by sending its rootlike haustoria in the host plant, the free ends of the dodder were brought into contact with trees affected with different mycoplasma diseases, by placing the dodderized periwinkle plant in the diseased trees.

In this way it is hoped that the dodder would form a living bridge between the diseased trees and the periwinkle, and thereby transmit the disease. By observing the symptoms of the different tree diseases in a host such as periwinkle it would be possible to determine their relationships.

Results are not yet consistent enough to be conclusive. But, according to the scientists, there appears to be a strong suggestion of mycoplasma-like bodies in the elm trees at the end.

The electron microscope makes possible the study of organisms normally invisible to the human eye.

Dodderized periwinkle plants, being examined here by Dr. Seliskar, may provide clues to the causes of tree disease.

disease research continues

Scientists at the Forest Service Laboratory are beginning further research into bunch diseases of mylberry, ironwood, hickory, and boxelder to determine their interrelationships. It is likely that these diseases may be caused by similar organisms, possibly even the same organisms in some cases.

What part the mycoplasmalike bodies play in causing these diseases is still not entirely known, but the cloak of mystery seems to be lifting, thanks to forestry research.